

Massachusetts Association of Blood Banks
Three Patients: Many Lessons

Christopher P. Stowell, MD, PhD
Massachusetts General Hospital
Harvard Medical School

Carole Calvert

- **CC:** 69 y.o. woman presents c/o pleuritic chest pain, dyspnea, light-headedness
- **PMH:** GERD, s/p hysterectomy for fibroids, 4 pregnancies, no transfusions
- **PE:** systolic ejection murmur
- **Angiography:** large mass in pulmonary artery, occluding left PA and narrowing right PA; elevated PA pressures

Carole Calvert – Blood Bank Work-up

Front				Back		Cell	IS	IAT
Anti-A	Anti-B	Anti-D	Du	A1 cells	B cells	ABS 1	4+	2+s
-	-	-	-	4+	4+	ABS 2	4+	2+s
						ABS 3	4+	2+s
						AC	-	-

Carole Calvert – Blood Bank Work-up

Front				Back		Cell	IS	IAT
Anti-A	Anti-B	Anti-D	Du	A1 cells	B cells	ABS 1	4+	2+s
-	-	-	-	4+	4+	ABS 2	4+	2+s
						ABS 3	4+	2+s
						AC	-	-
						i (adult)	2+	2+s
						i (cord)	1+	2+
						Tj(a-)	2+	3+

Carole Calvert – Blood Bank Work-up

Front				Back				
Anti-A	Anti-B	Anti-D	Du	A1 cells	B cells	H Lectin	Anti-Le(a)	Anti-Le(b)
-	-	-	-	4+	4+	-	1+	-

Carole Calvert – Blood Bank Work-up

- Conclusion: Bombay phenotype - O_h
 - RBC H – deficient
 - non-secretor
 - anti-H – reactive RT and IAT
- Surgery planned likely to require RBC transfusion
- Source for RBC
 - Autologous – not enough time
 - Siblings – healthy younger brother Bombay phenotype
 - American Rare Donor registry (AABB and ARC)

Carole Calvert – Crossmatches

Front				Back		Donor Unit	IS	IAT
Anti-A	Anti-B	Anti-D	Du	A1 cells	B cells	Rare Donor Registry O _h	-	-
-	-	-	-	4+	4+	Brother (O _h unit 1)	-	-
						Brother (O _h unit 2)	-	-

Carole Calvert – Follow-up

- **Procedure:** L thoracotomy, L pneumonectomy, resection of tumor and PA reconstruction
- **Hemoglobin levels:**
 - Pre-op: 11.7 g/dL
 - Intraop: 6.4 g/dL
 - Post-op: 8.8 g/dL
- **RBC transfusion:** 2 unit intra-op, 1 unit post-op
- **Discharge:** post-op day 8, hemoglobin 11.4 g/dL

Romain Dubois

- **CC:** 52 y.o. man presents with unstable angina
- **PMH:** history of stable angina and non-Q-wave MI, hypercholesterolemia, benign prostatic hypertrophy, no transfusions
- **PE:** EKG shows T wave inversions
- **Cardiac catheterization:** 3 vessel disease with 73% occlusion LAD
- **Type and Screen:** !!! Comes with a history of being 'Bombay' phenotype

Romain Dubois - Blood Bank Work-up

Front				Back		Cell	IS	IAT
Anti-A	Anti-B	Anti-D	Du	A1 cells	B cells	ABS 1	4+	4+
2+	-	4+	n.a.	4+	4+	ABS 2	4+	4+
						ABS 3	4+	4+
						AC	-	-

Romain Dubois - Blood Bank Work-up

Front				Back				
Anti-A	Anti-B	Anti-D	Du	A1 cells	B cells	H Lectin	Anti-Le(a)	Anti-Le(b)
2+	-	4+	n.a.	4+	4+	-	2+	-

Romain Dubois- Blood Bank Work-up

- Conclusion: Para-Bombay phenotype - A_h
 - RBC H – weak
 - non-secretor
 - anti-H – reactive RT and IAT
- Procedure *un*-likely to require RBC transfusion

Romain Dubois - Follow-up

- **Procedure:** PTCA and stenting of LAD performed successfully
- **Hemoglobin levels:**
 - Pre-op: 14.5 g/dL
 - Post-op: 13.4 g/dL
- **RBC transfusion:** none
- **Discharge:** post-procedure day 2, hemoglobin 13.5 g/dL

Nicole Forrestier

- **CC:** 54 y.o. woman presents c/o severe headache, nausea and vomiting
- **PMH:** hypertension, migraines
- **PE:** no neurological abnormalities
- **CT scan:** subarachnoid hemorrhage in basal cisterna
- **Angiography:** basilar artery and posterior cerebral artery aneurysms
- **Type and Screen: !!!!**

Nicole Forrestier - Blood Bank Work-up

Front				Back		Cell	IS	IAT
Anti-A	Anti-B	Anti-D	Du	A1 cells	B cells	ABS 1	-	w+
2+	-	3+	n.a.	2+	3+	ABS 2	-	w+
						ABS 3	-	w+
						AC	-	-

Nicole Forrestier - Blood Bank Work-up

Front				Back				
Anti-A	Anti-B	Anti-D	Du	A1 cells	B cells	H Lectin	Anti-Le(a)	Anti-Le(b)
3+	-	3+	n.a.	2+	3+	-	2+	-

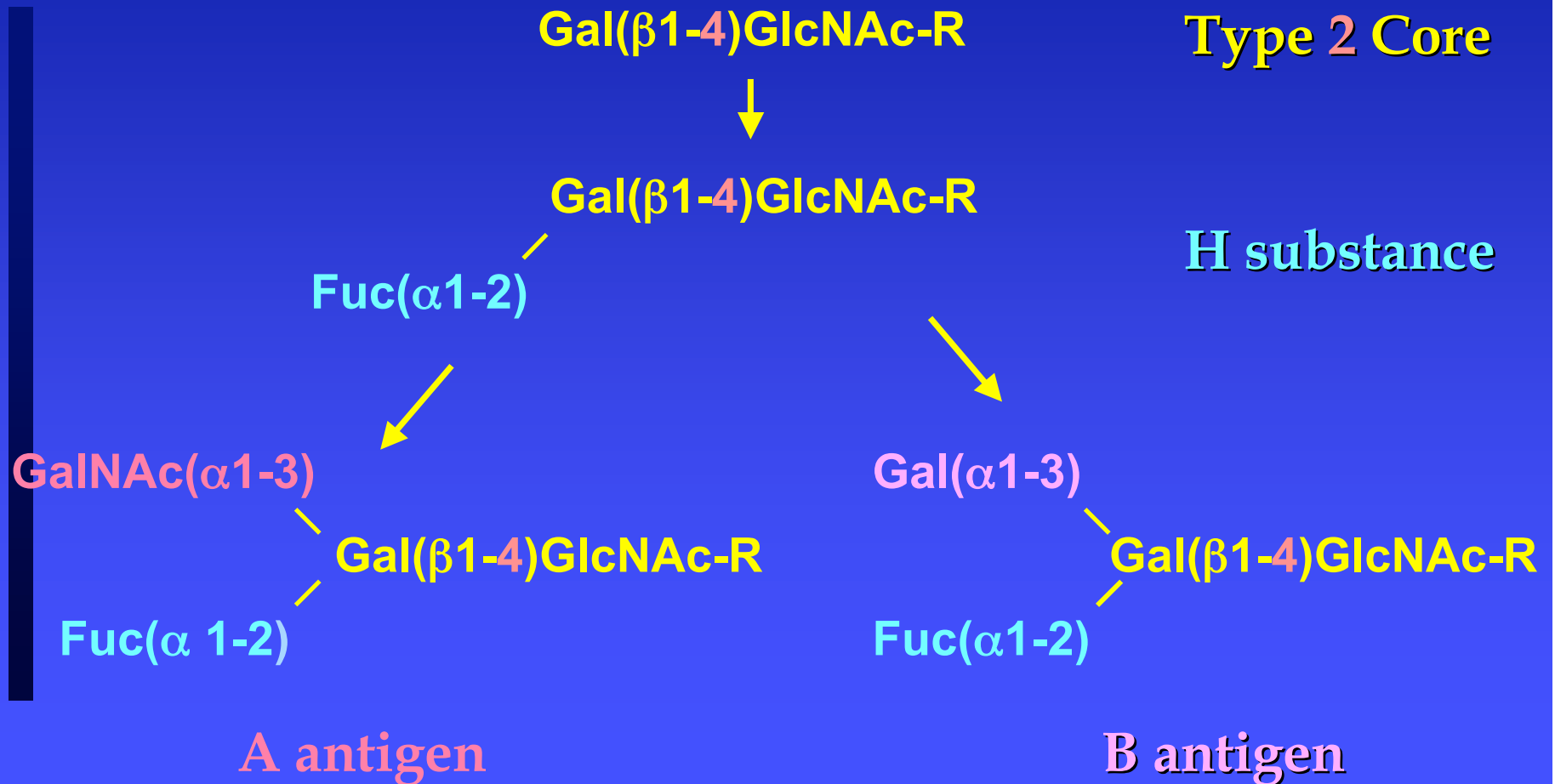
Nicole Forrestier – Blood Bank Work-up

- **Conclusion:** Para-Bombay phenotype - A_h
 - RBC H – weak
 - non-secretor
 - anti-H (-HI?) – reactive IAT but weak
- Procedure *un*-likely to require RBC transfusion

Nicole Forrestier – Follow-up

- **Procedure:** femoral artery catheterization and endovascular coiling of both aneurysms
- **Hemoglobin levels:**
 - Pre-op: 9.1 g/dL
 - Post-op 9.0 g/dL
- **RBC transfusion:** none
- **Discharge:** post-procedure day 5, hemoglobin 9.8 g/dL

Synthesis of ABH Antigens



Concept

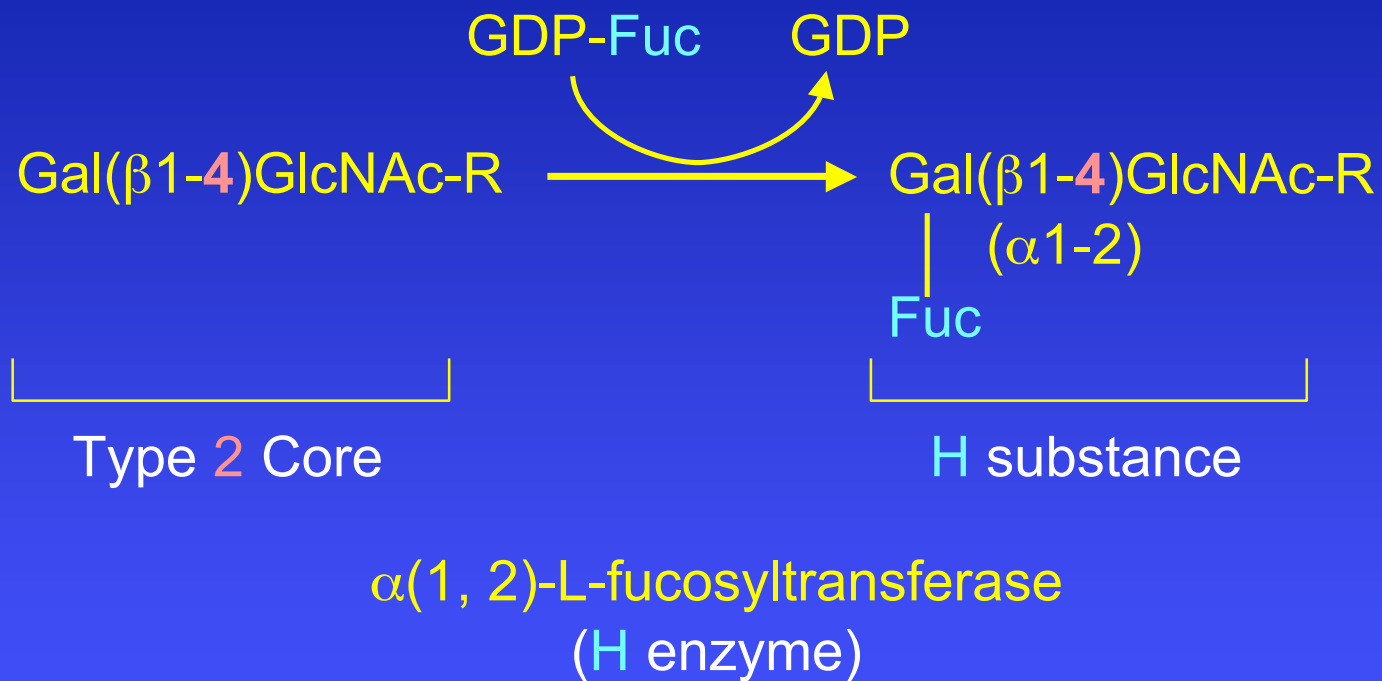
- Protein Antigens - structure is directly controlled by gene

gene → transcript → antigen

- Carbohydrate Antigens - structure is indirectly controlled by gene

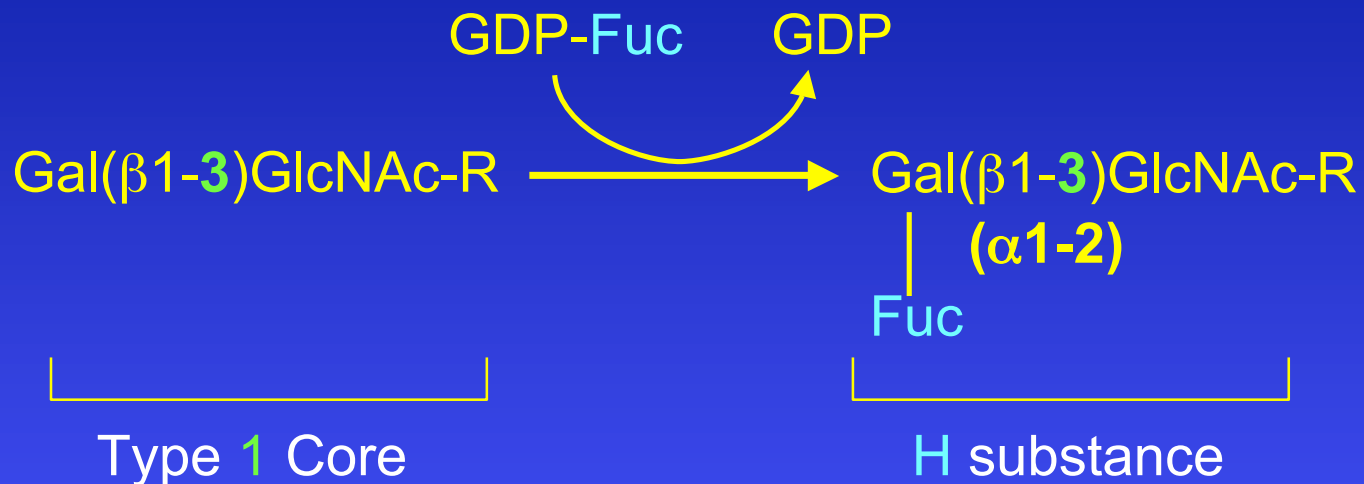
gene → transcript → glycosyltransferase → antigen

Synthesis of H - H Gene



H enzyme expressed in mesodermal tissues - e.g., RBC

Synthesis of H - *Se* Gene



$\alpha(1, 2)\text{-L-fucosyltransferase}$
(*Se* enzyme)

Se enzyme expressed in endodermal tissue - e.g., glandular epithelia

Secretor “System”

Se allele is phenotypically dominant over **se**.
People who are **Se/-** make (secrete) ABH substance. They are called “secretors.”

se allele is phenotypically recessive to **Se**. People who are **se/se** (20%) do not make (or secrete) ABH substance. They are called “non-secretors”

H and Secretor

<u>Gene</u>	<u>Enzyme</u>	<u>Chromosome</u>	<u>Substrate</u>
<i>H (FUT1)</i>	Fuc TI	19q13.3	Type 2
<i>SE (FUT2)</i>	Fuc TII	19q13.3	Type 1
Sec 1	Ψgene	19q13.3	none

Revised (Two Locus) Model for Interaction of *H* and *Se*

H expressed in RBC

Se expressed in epithelia

Genotypes		Phenotypes	
<i>H/h</i>	<i>Se/se</i>	RBC	Secretions
<i>H/</i>	<i>Se/</i>	H	secretor
<i>H/</i>	<i>sese</i>	H	nonsecretor
<i>hh</i>	<i>sese</i>	Bombay	nonsecretor
<i>hh</i>	<i>Se/</i>	Bombay	secretor

The 'Classic' Bombay Phenotype - O_h

The story starts with three gentlemen from Bombay (Mumbai)...

... who have no **A**, **B** or **H** antigens on their RBC

... who also have no **A**, **B** or **H** in their secretions (just Le^a)

... and who make potent anti-**H** isoagglutinin (+ anti-**A** and anti-**B**)

H and *h* 'Classic' Bombay Alleles

H Allele

T 725

Leu 242

h Allele

G 725

Arg 242

non-functional *H* enzyme

The 'Réunion' Bombay (or Para-Bombay) Phenotype

People with this phenotype...

... have *weak* expression of **A**, **B** or **H** antigens on their RBC

... have no **A**, **B** or **H** in their secretions (just Le^a)

... make weak anti-**H** (+ anti-**A** and anti-**B**)

... and know their wines

H and *h* 'Réunion' Alleles

H Allele

C 349

His 117

h Allele

T 349

Tyr 117

weakly functional *H* enzyme

Types of *h* Alleles

Mis-sense mutations – snp results in substitution of one amino acid for another
e.g. ‘Classic’ and ‘Réunion’ Bombay

Nonsense mutations – snp or deletion leads to premature ‘stop’ codon by substitution of one amino acid for another or by frameshift

Missense Mutations in the H gene (FUT 1)

Nucleotide Change	Amino Acid Change	H enzyme Activity	Phenotype	Population
725 T→G	242 Leu→Arg	none	'Classic' Bombay	Indian
349 C→T	117 His→Tyr	weak	'Réunion' Bombay	European Réunion Is.
461 A→G	154 Tyr→Cys	none	Bombay	European
658 C→T	220 Arg→Cys	none	Para-Bombay (Se)	Taiwan
917 C→T	305 Thr→Ile	weak	Para-Bombay	Brazil (Amerindian)
1042 G→A	348 Glu→Cys	weak	Para-Bombay	Taiwan Japan

Nonsense Mutations in the *H* gene (*FUT 1*)

Nucleotide Change	Amino Acid Change	H enzyme Activity	Phenotype	Population
421 A→G	140 Leu→stop	none	Bombay	European
538 C→T	180 Gln→stop	weak	Para-Bombay	Israel
547 ΔAG	182 Arg→frmsht	weak	Para-Bombay	Taiwan
695 G→A	232 Try→stop	none	Bombay	Japan
969 ΔCT	323 Val→frmsht	none	Bombay	European
990 ΔG	330 Asn→frmsht	weak	Para-Bombay	Japan

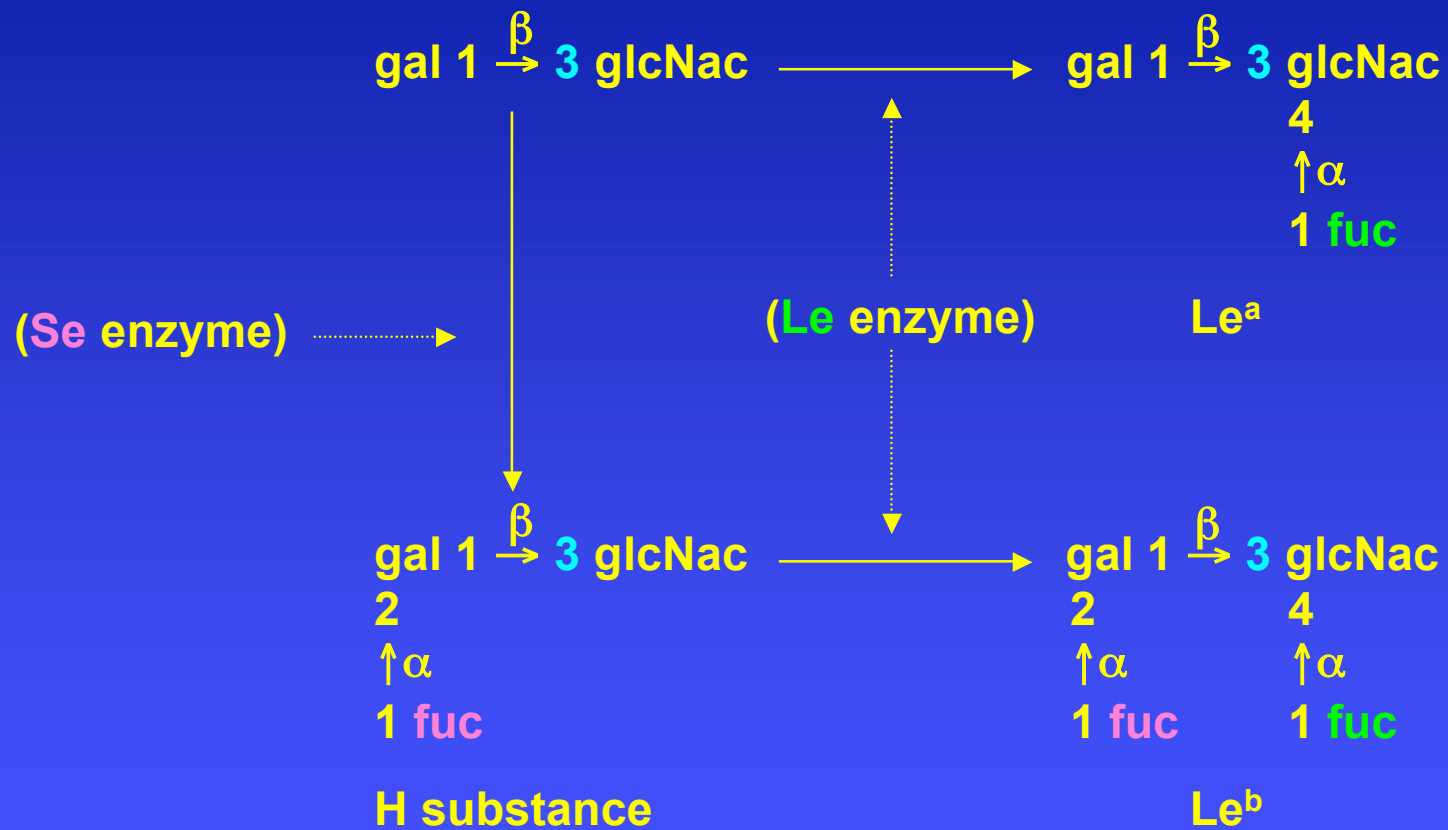
Functional Categories: *FUT 1* Alleles

Allele	H Enzyme Activity	ABH on RBC	Examples
<i>H</i>	normal	normal	you
<i>h</i> 'weak'	reduced	weak 'Para-Bombay'	Réunion Phenotype
<i>h</i>	absent	absent 'Bombay'	'Classic' Bombay

Mutations in the *Se* gene (*FUT 2*)

Nucleotide Change	Amino Acid Change	Se Enzyme Activity	Phenotype	Population
gene deletion <i>se</i> ^{del}	no enzyme	none	'Classic' Bombay (<i>se</i>)	Indian
<i>Sec1/FUT2</i> fusion gene	no enzyme	none	non-secretor	Japan, Taiwan
385 C→T	129 Ile→Phe	weak	secretor Le(a+b+)	Japan, Taiwan (common)
428 A→G	140 Try→stop	none	non-secretor	European, Réunion (common)
664 C→T	222 Arg→Cys	none	non-secretor	New Guinea
688 ΔGTC	230 ΔVal	none	non-secretor	Philippines

Se and Lewis Enzymes



Functional Categories: *FUT 2* Alleles

Allele	Se Enzyme Activity	ABH in Secretions	Examples
Se	normal	Normal ($\pm Le^b$)	80% of you
Se^{wk}	reduced	weak ($\pm Le^a+Le^b$)	common East Asians
se (se^{del})	absent	absent ($\pm Le^a$)	20% of you (Classic Bombay)

Doing the math...

	H	h^{wk}	h
Se	✓	✓ Para- Bombay	✓ Para- Bombay
Se^{wk}	✓		
se	✓	✓ Para- Bombay	✓ Bombay

Sorting it out...

H gene	Se gene	Example	Population
<i>h</i>	Se	<i>h</i> 658 C→T	Taiwan
	Se^{wk}		
	se	<i>h</i> 785 G→A	Europe
	se^{del}	<i>h</i> 725 T→G	Classic Bombay
<i>h^{wk}</i>	Se	<i>h^{wk}</i> 460 T→C	Japan, Taiwan
	Se^{wk}		
	se	<i>h^{wk}</i> 349 C→T	Réunion Phenotype

Mechanisms of Weak ABH Expression

In non-secretors

- H enzyme with low activity
 - 349 C→T Réunion phenotype
 - 1042 G →A (Japan and Taiwan)

In secretors

- H enzyme with low activity
 - 460 T →C (Japan)
- No H enzyme activity but RBC adsorption of secreted ABH glycolipids (Secretor phenotype)
 - 658 C →T (Taiwan)

Putting it all together...

Genotype		ABO Antigens		Symbols	Plasma		
<i>H</i>	<i>Se</i>	RBC	Scrtns		Anti-A	Anti-B	Anti-H
<i>hh</i>	<i>sese</i>	0	0	O_h or O_h^O , O_h^A ...	+	+	+
<i>h^{wk}h^{wk}</i> <i>h^{wk}h</i>	<i>sese</i>	0/wk	0	O_h , A_h ...	per ABO*	per ABO	+
<i>h^{wk}h^{wk}</i> <i>h^{wk}h</i>	<i>Se_</i>	0/wk	+	O_{hm}^O , O_{hm}^A ...	per ABO	per ABO	Anti-HI
<i>hh</i>	<i>Se_</i>	0/wk	+				

* A_h may have anti- A_1

The Received Wisdom

RBC H deficient, non-secretors ('Bombay') and RBC H weak, non-secretors, make potent anti-H (+ anti-A and anti-B)

RBC H deficient or weak, secretors make weak anti-HI (+ anti-A or anti-B per genetic blood group)

Anti-'H' in H-deficient Phenotypes

13 RBC weak ABH probands

4 h^{wk} alleles – H transferase activity 2-10%

	Secretors n=11	Non- secretors n=2
Anti-H	5	0
Anti-HI	6	2

* Kaneko M, *et al. Blood* 1997;90:839-49.

Anti-'H' in H-deficient Phenotypes

Proband	A/B antigen	H antigen	Le type	Genotype	Anti-H
1*	no	no	Le ^b	<i>h/Se</i>	mod
2*	no	no	Le ^a	<i>h/se</i>	strong
3*	weak A	no	Le ^b	<i>h or h^{wk} /Se</i>	4C RT IAT
4*	no	weak	Le ^a	<i>h^{wk} /se</i>	18C opt
5*	no	no	Le ^a	<i>h/se</i>	strong
6**	weak A B	weak	Le ^b	<i>h or h^{wk} /Se</i>	+
7**	weak A	weak	Le ^b	<i>h or h^{wk} /Se</i>	+

* Storry J, *et al. Transfusion* 2006;46:2149-55.

** Yan L, *et al. Transfusion* 2005;45:725-30.

Our Patients

Patient	A/B present	H present	Anti-A	Anti-B	Anti-H	Le type	Genotype
C.C.	no	no	yes	yes	strong	Le ^a	<i>h</i> / <i>se</i> O _h [?]
R.D.	wk A	no	yes	yes	strong	Le ^a	<i>h</i> ^{wk} / <i>se</i> A _h
N.F.	wk A	no	weak	yes	weak	Le ^a	<i>h</i> ^{wk} / <i>se</i> A _h

RBC Survival in RBC H Deficient Secretors with anti-HI

18 subjects O_{hm}^B (H deficient secretors)

10 no anti-H or-HI at 37C or IAT

6 anti-HI at IAT

2 anti-H at IAT

^{51}Cr labeled O or B RBC injected into 3 different O_{hm}^B subjects

Subject	Antibody at IAT		$T_{1/2}$
	Anti-B	Anti-HI	
1	0	0	< 60 min
2	+	+	< 60 min
3	0	+	< 60 min

Lin-Chu M, Broadberry RE. *Br J Haematol.* 1990;75:568-72

RBC Survival in RBC H Deficient Secretors with anti-HI

⁵¹Cr labeled B RBC (130 mL) injected into RBC H deficient (*hh*, *B-*) secretor – strong anti-A, weak anti-B, weak anti-HI

Time	% ⁵¹ Cr survival (expect)	DAT	Hapto (mg/dL)	Bili (D/T) (mg/dL)	HgB (g/dL)
24 h	93 (96)	Neg	295	0.1/0.4	13.3
48 h	82 (94)	Neg	218	0.1/0.3	13.2
28 d	30 (76)	Neg	195	0.1/0.3	12.8

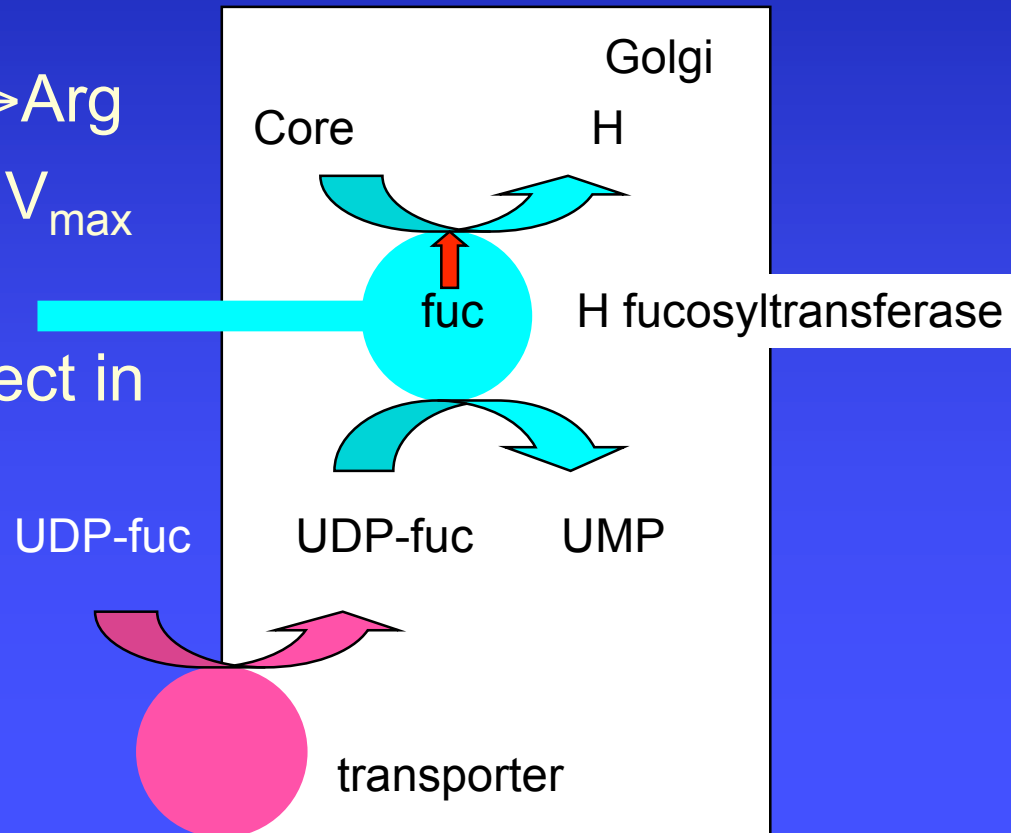
Lin-Chu M, Broadberry RE. *Br J Haematol.* 1990;75:568-72

Yet another mechanism for the Bombay phenotype: Leukocyte Adhesion Deficiency Type II (LAD II)

- 5 patients (!)
- All boys – 4 Arabs, 1 Turk
 - Recurrent infections
 - Psychomotor and growth retardation
 - Absence of sialyl Lewis X antigen (Le^a on type 2 core)
 - Bombay RBC (and must be non-secretors)

Yet another mechanism for the Bombay phenotype: Leukocyte Adhesion Deficiency Type II (LAD II)

- Basic defect – reduced function of GDP-fucose transporter
- C→G; 308 Thr→Arg
- Transporter low V_{max}
- Generalized defect in fucosylation



Methodological Problems Studying H-deficient Phenotypes

- Definition of AB-deficient RBC based on...?
 - Conventional anti-A and anti-B typing reagents \pm anti-A,B
 - Conventional AB typing reagents with ficin-treated cells
 - Adsorption elution
- Definition of H deficient RBC based on...?
 - H lectin (*Ulex europaeus*)
 - Human anti-H

Methodological Problems Studying H-deficient Phenotypes

- Definition of anti-'H' significance based on...?
 - 4C, RT, 37C
 - IS, IAT
 - titer
 - Lewis typing (secretor status)
- Definition of anti-'H' specificity based on...?
 - reagent cells: O_I, O_i, O_h